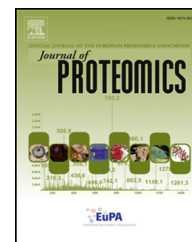


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Review

The Spanish biology/disease initiative within the human proteome project: Application to rheumatic diseases☆

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ABSTRACT

The Spanish Chromosome 16 consortium is integrated in the global initiative Human Proteome Project, which aims to develop an entire map of the proteins encoded following a gene-centric strategy (C-HPP) in order to make progress in the understanding of human biology in health and disease (B/D-HPP). Chromosome 16 contains many genes encoding proteins involved in the development of a broad range of diseases, which have a significant impact on the health care system. The Spanish HPP consortium has developed a B/D platform with five programs focused on selected medical areas: cancer, obesity, cardiovascular, infectious and rheumatic diseases. Each of these areas has a clinical leader associated to a proteomic investigator with the responsibility to get a comprehensive understanding of the proteins encoded by Chromosome 16 genes. Proteomics strategies have enabled great advances in the area of rheumatic diseases, particularly in osteoarthritis, with studies performed on joint cells, tissues and fluids.

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† In memoriam of Juan Pablo Albar.

Biological significance

In this manuscript we describe how the Spanish HPP-16 consortium has developed a B/D platform with five programs focused on selected medical areas: cancer, obesity, cardiovascular, infectious and rheumatic diseases. Each of these areas has a clinical leader associated to a proteomic investigator with the responsibility to get a comprehensive understanding of the proteins encoded by Chromosome 16 genes. We show how the Proteomic strategy has enabled great advances in the area of rheumatic diseases, particularly in osteoarthritis, with studies performed on joint cells, tissues and fluids.

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Contents

1. The Spanish Hpp consortium	0
2. Chromosome 16 and disease	0
3. The Spanish biology/disease platform	0
4. SpHPP B/D initiative on rheumatic diseases	0
5. Proteomic screening of the osteoarthritic joint	0
6. Development of the Hpp pillars in osteoarthritis research	0
7. Conclusions	0
Transparency Document	0
Conflict of interest disclosure	0
Acknowledgments	0
References	0

1. The Spanish Hpp consortium

Since its foundation, the Spanish Human Proteome Project consortium (SpHPP) is structured on two platforms on the basis of ProteoRed-ISCI network (Fig. 1). This consortium is integrated in the global initiative Human Proteome Project [1], which aims to develop an entire map of the proteins encoded following a gene-centric strategy (C-HPP) [2] in order to make progress in the understanding of human biology in health and disease (B/D-HPP).

The SpHPP project was conceived on a multi-centric configuration, assuming the standards and integration procedures already available in ProteoRed-ISCI (http://www.proteored.org), which are encompassed with the HUPO initiatives. Within this consortium, the Spanish C-HPP platform aims to identify and characterize previously known and unknown proteins of Chromosome 16, whereas the B/D-HPP platform aims the cooperation of basic researchers with clinical groups belonging to networks of the Spanish Institute of Health (ISCI): CIBERS (Networks of Biomedical Research Centers) and RETICS (Thematic Networks of Cooperative Health Research). This will facilitate not only the comprehensive study of the products of the 836 protein-coding genes in Chromosome 16 in diverse sample types (cells, tissues or biofluids), but also their possible role in disease pathogenesis, as well as their putative usefulness for the development of diagnosis, prognosis and treatment strategies.

2. Chromosome 16 and disease

Alterations in Chromosome 16, which comprises near 900 proteins, have been linked to several different disorders such

as obesity, autism, neurodegenerative diseases, cancer, thalassemia or spondyloarthropathies. Genetics Home Reference (http://ghr.nlm.nih.gov) lists 58 conditions that are related to genes on Chromosome 16. These conditions include not only common hemoglobinopathies such as alpha-thalassemia, but also different types of cancers, inflammatory bowel diseases such as Crohn and a number of metabolism-related syndromes. A comprehensive proteomic analysis of Chr. 16 proteins will be highly valuable for a better understanding of these pathologies.

3. The Spanish biology/disease platform

With the aim of coordinating all those efforts related with biology and disease driven projects within the HPP in Spain, the SpHPP consortium has developed a BD platform with a manager and five programs focused in selected medical areas, based on those genes located in the Chromosome 16 (Fig. 2): cancer, obesity, cardiovascular, infectious and rheumatic diseases. Each of these areas has a clinical leader (oncologist, endocrinologist, cardiologist, internal medical specialist and rheumatologist), and are co-chaired by a ProteoRed-ISCI member.

Clinicians are associated to proteomic investigators with the responsibility to get a comprehensive understanding of the proteins encoded by the genes and their relationship with disease. Based on the genes located in the Chromosome 16, ten diseases (colon cancer, brain cancer, breast cancer, obesity, atherosclerosis, candidiasis, rheumatoid arthritis and osteoarthritis) have been selected to acquire significant medical benefits, including improved capabilities for early detection and diagnosis, therapeutic development and monitoring and personalized healthcare programs.

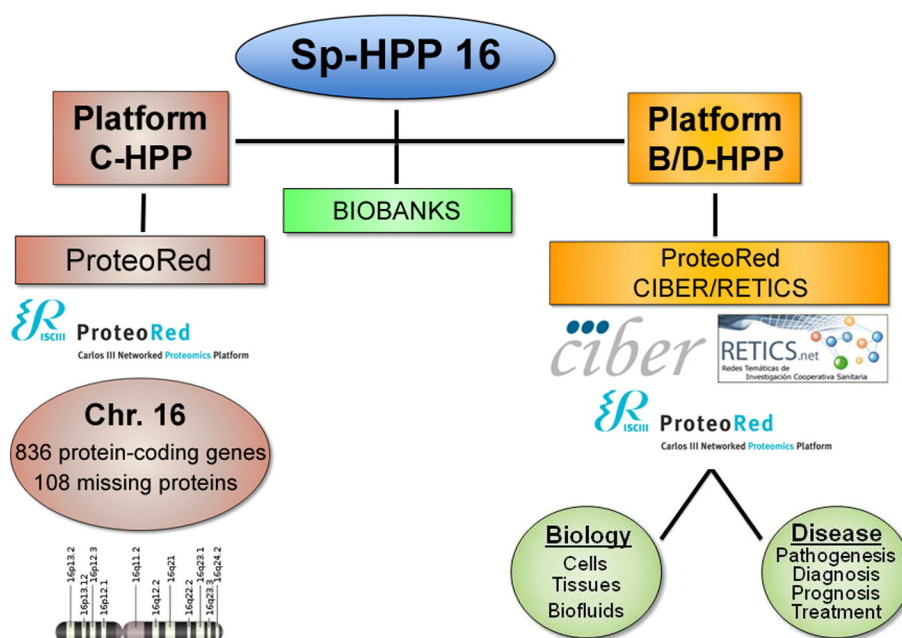


Fig. 1 – The SHPP-16 consortium. The C-HPP is structured on the basis of ProteoRed network, and its main objective is the identification and proteomic characterization of previously known and unknown proteins encoded on Chromosome 16. The B/D-HPP platform aims the cooperation of ProteoRed researchers with clinical groups belonging to networks of the Spanish Institute of Health (ISCIII): CIBER and RETICS. This will facilitate not only the comprehensive study of proteins encoded on the Chromosome 16 in diverse sample types (cells, tissues or biofluids), but also their possible role in disease pathogenesis, or their putative usefulness for the development of diagnosis, prognosis and treatment strategies.

The Clinical Research that is essential to validate the proposed biomarkers on each medical area will be carried out using the national clinical networks of the Spanish Health Administration (National Institute of Health, ISCIII) such as the CIBER on obesity (CIBEROBN) and different RETICS: Cardiovascular Network (RIC), Cancer National Network (RTICC), Infectious Pathologies Network (REIPI) and National Network of Inflammation and Rheumatic Diseases (RIER).

Each medical area will focus on one to three specific diseases (Fig. 2). As the quality of biological samples is a critical point to obtain high confidence results in the HPP project, the Spanish National Biobank Network (also belonging to the Carlos III National Health Institute) has been involved in the collection and storage of all selected biological fluids and tissues that are needed to carry out clinical research in the B/D-HPP platform, and will be the provider of clinical samples to the project.

4. Sphpp B/D initiative on rheumatic diseases

Among the five medical areas of the Spanish B/D initiative, this article will then focus on describing the work performed in the area of rheumatic diseases. Rheumatism is a non-specific term used to describe any painful disorder affecting the loco-motor system including joints, muscles, connective tissues and soft tissues around the joints and bones. Rheumatologic disorders include highly prevalent pathologies such as osteoarthritis, inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis or spondyloarthropathies, and autoimmune diseases

such as systemic lupus erythematosus (SLE), Behçet's syndrome or sarcoidosis.

There are several genes encoded in Chromosome 16 with a possible reported role in the pathogenesis of a number of rheumatologic disorders, which can be classified into three groups of diseases (Table 1): A, age-related rheumatic pathologies; B, inflammatory arthritis; and C, autoimmune disorders.

Osteoarthritis (OA, Group A), is the most frequent arthropathy, ultimately affecting more than 10% of the population and being the leading cause of permanent work incapacity. It is characterized by the progressive degradation of hyaline articular cartilage and is associated with aging [3]. Among inflammatory arthritis (Group B), Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of undetermined etiology involving primarily the synovial membranes and articular structures of multiple joints. It is associated with progressive and severe disability and early death, mainly due to not only increased cardiovascular disease but also to many other comorbidities. A number of proteomic studies have been performed to elucidate the pathogenic mechanisms of this disease, and also to search for novel biomarkers useful for therapy monitoring [4,5]. Apart from RA, other relevant arthritis such as psoriatic arthritis (PA) or spondyloarthropathies (SpAs) should be taken into account. In this sense, a direct link between mutations in the NOD2 gene (located on chr. 16) and Crohn's disease has been recently found [6]. Interestingly, this disease has a probable autoimmune origin and is associated with the development of SpAs. Finally, other characteristic autoimmune disorders will be the subject of our studies (Group C, Table 1). Among these, SLE is an autoimmune connective

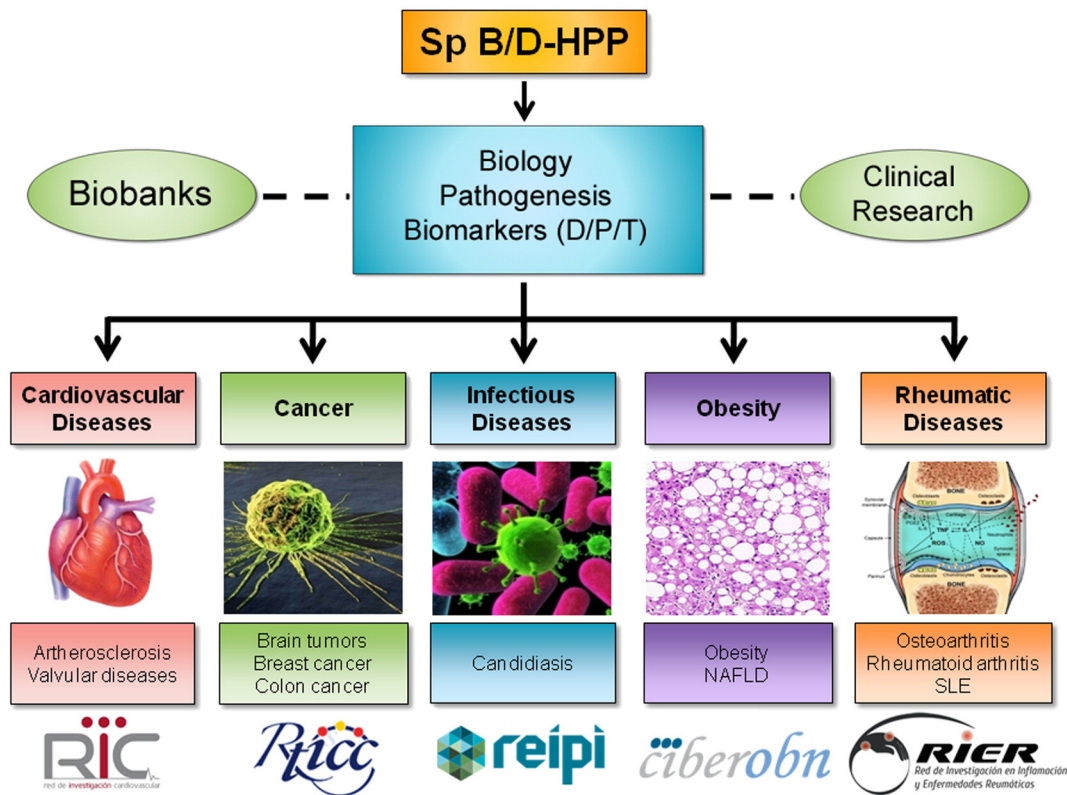


Fig. 2 – The Spanish B/D-HPP platform. Five medical areas have been selected, based on the genes located in the Chromosome 16. These are led by a clinician and co-chaired by a ProteoRed member. Each medical area will focus on one to three specific diseases (NAFLD: non-alcoholic fatty liver disease; SLE: systemic lupus erythematosus). Clinical samples needed to perform the experiments will be stored and provided by the Spanish National Biobank Network. Clinical research to validate the proposed disease biomarkers in each medical area will be carried out using the national clinical networks of the Spanish Health Administration (Carlos III National Institute of Health, ISCIII): CIBER (CiberOBN) and RETICS (RIC, RTICC, REIPi and RIER).

tissue disease of unknown origin affecting virtually all organ systems, with no specific cause but a number of environmental triggers and a number of genetic susceptibilities. The

Table 1 – Genes encoded on Chromosome 16 that are related with rheumatic diseases.

Disease	Genes in Chromosome 16
A. Aging Osteoarthritis (OA)	Heparan sulfate synthases (HS3ST2, 4 and 6) Chondroitinsulfatase (GALNS) MMPs (MMP2, 15 and 25) CLUAP1 Proteins related with the mitochondrial function
B. Arthritis Rheumatoid arthritis (RA) Psoriatic arthritis Spondyloarthropathies	ILs (IL-17, 27, 32 and 34) ILRs (IL4R and 21R) TNFRs (TNFRSF12A and 17) NOD2
C. Autoimmune disorders Systemic lupus erythematosus (SLE) Beçet's disease Sarcoidosis	CIITA Integrin precursors (ITGA-D, L, M and X) Complement components (C1QTNF8)

understanding of the genetic basis of SLE has been rapidly advanced using large-scale, case-control, candidate gene studies as well as genome-wide association studies during the last years [7]. One of the best known associations was found between SLE susceptibility and ITGAM or ITGAM-ITGAX region [8], which encodes integrin alpha-M (also known as CD11b or complement receptor 3), the α -chain of the $\alpha\text{M}\beta 2$ integrin. Both ITGAM and ITGAX are localized in Chromosome 16.

5. Proteomic screening of the osteoarthritic joint

As a starting point for the study of rheumatic diseases from the perspective of the HPP project, our group has performed in the recent years several proteomic analyses on cells, tissues and biofluids from the osteoarthritic joint, in order to increase the knowledge about the pathogenesis of this disease [9], and also to discover novel OA biomarker candidates [10] (Fig. 3).

The first studies were carried out on human articular chondrocytes (which are the only cell type resident in mature cartilage) in primary culture. These works led to the definition of a panel of chondrocyte intracellular proteins that are quantitatively altered in osteoarthritic cells compared to healthy controls [11] (including chaperones and glycolytic proteins), and also to the characterization of the chondrocyte's

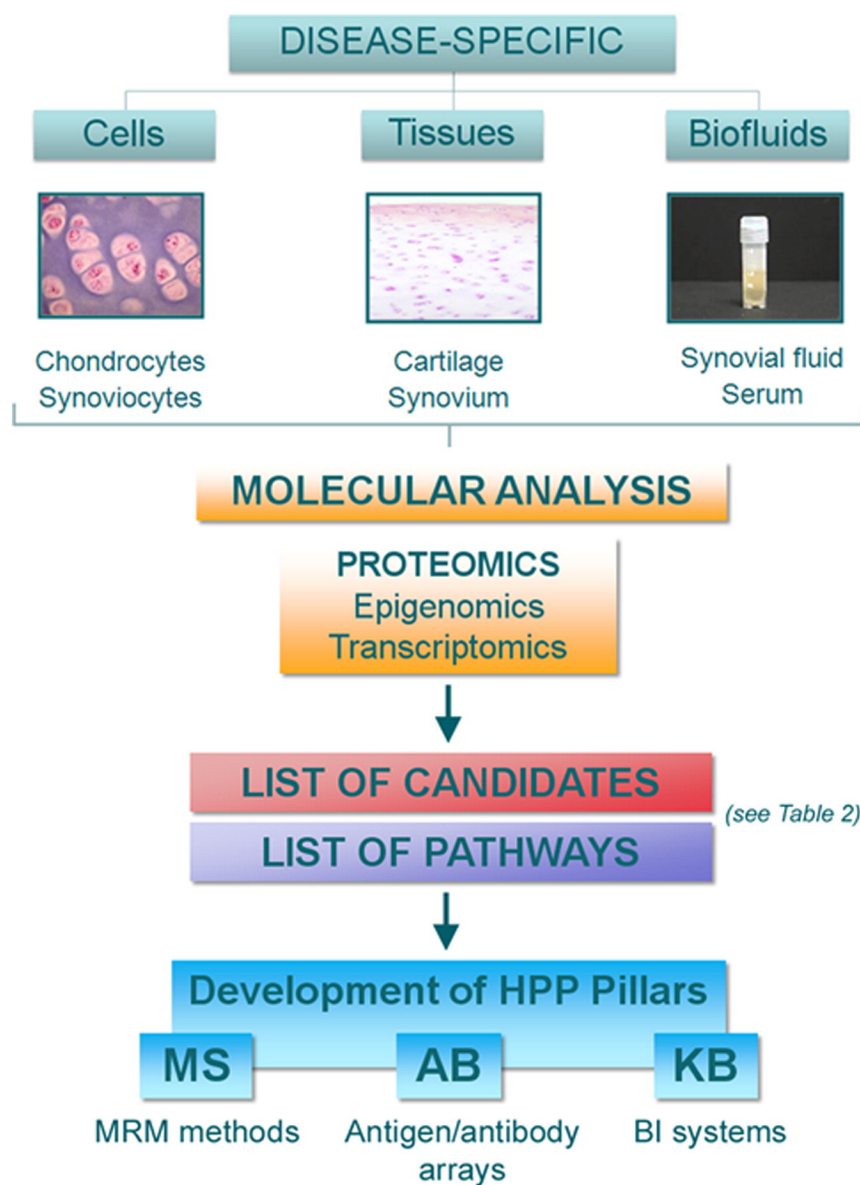


Fig. 3 – Workflow of the Spanish B/D-HPP initiative on rheumatic diseases. Proteomic screening on disease-specific cells, tissues and biofluids has led to the definition of a series of candidates related with different biological pathways (an example of ten of these candidates is shown in Table 2). The development of the three HPP pillars is currently being performed: MS, through the design of targeted MRM methods and its application on clinical cohorts; AB, through the fabrication of antigen and antibody arrays and its application on clinical cohorts; and KB, through the creation of business intelligence (BI) systems for knowledge management.

response against hypoxic conditions [12], proinflammatory mediators such as IL-1 β or TNF- α [13], or drugs intended for the treatment of osteoarthritis [14]. Apart from these studies on whole chondrocytic proteins, proteomic profiling of chondrocyte mitochondria was also carried out [15,16]. These latter works unraveled the mitochondrial dysregulation that occurs in osteoarthritis, which has been previously reported and was further explored in other studies [17,18]. Finally, additional assays were performed using the SILAC approach to investigate the protein profiles of the subset of chondrocyte secreted proteins (secretome) in response to IL-1 β [19] or anti-OA drugs [20,21], considering the essential role of this cell in synthesizing the cartilage extracellular matrix and

maintaining its proper turnover. All these studies provide key information about the mechanisms participating in osteoarthritis pathogenesis, and also those driven in cartilage cells by either proinflammatory or therapeutic agents.

Furthermore, proteomic analyses on human articular cartilage have also been performed. This highly specialized tissue has an organized architecture and is composed mainly (95–98%) of a dense extracellular matrix (ECM) including collagens and proteoglycans that make the protein extraction very difficult. In spite of this, some studies have compared the protein profiles of healthy and osteoarthritic tissue [22,23]. Furthermore, other reports have focused on the peptides or proteins released to the culture medium from cartilage explants.

Most of these works showed the effect on human tissue of metalloproteases [24] or cytokines [25] as in vitro models of disease. Very recently, our group has conducted an analogous study, but comparing the protein profiles released from cartilages of healthy donors, macroscopically intact tissue of OA patients, and damaged tissue from the same individuals [26].

The proteins depicted in the above-mentioned works may also have biomarker utility, as they are altered in chondrocytes or cartilage as a consequence of the disease or the cellular response to an outer stimulus. In this sense, great efforts are being made in the recent years for the search of novel biomarkers useful for diagnosis, prognosis and monitorization of therapeutic responses in osteoarthritis [27]. Ideally, biochemical markers are derived from body fluids where they can easily be measured and monitored, such as serum, plasma or urine, and also from joint-specific samples such as synovial fluid. Using serum cohorts from OA patients and controls, some studies have identified differential proteins with putative biomarker value by SELDI-TOF [28,29] and also following an iTRAQ-based approach [30]. Alternatively, synovial fluid provides the advantage of being located where the disease occurs, thus containing higher concentrations of cartilage degradation products compared to other body fluids, but have the drawback of being more difficult to obtain (especially from healthy donors or at early disease stages). Therefore, some proteomic studies have analyzed OA synovial fluid using samples from rheumatoid arthritis patients as comparator group [31,32] and only two works have addressed differences between OA and healthy controls by shotgun proteomics [33,34], both using a small amount of samples for the analysis.

6. Development of the Hpp pillars in osteoarthritis research

All those shotgun studies reported above have led to the definition of a series of proteins related with the pathogenesis

of osteoarthritis, or putative biomarker candidates for early diagnosis, prognosis and development of drugs for OA treatment. As an example of this, Table 2 shows the top 10 proteins most commonly identified in our shotgun studies as altered in osteoarthritis disease, either in human articular chondrocytes [19], articular cartilage [26], synovial fluid [4] or serum [30]. Interestingly, while some of these proteins have been already proposed as OA biomarkers and are currently undergoing qualification studies (such as COMP [35] or YKL-40 [36]); most of them are novel biomarker candidates for OA. Multiplexed evaluations of these panels of proteins are needed to advance further in the OA biomarker pipeline [10]. For this task, targeted proteomics technologies are highly valuable, not only because of their capacity for simultaneously analyzing multiple analytes, but also because of their potential applicability in high-throughput studies such as clinical trials [37]. Active research will be the key for facilitating the categorization of OA biomarkers using the BIPEDS classification scheme that has been developed as framework for communication in the field [38,39]: burden of disease, investigative, prognostic, efficacy of intervention, diagnostic or safety markers. Therefore, attention should now be focused on the implementation of the three pillars of HPP [1] (mass spectrometry, affinity proteomics and knowledgebase) in order to develop improved methods and technologies that facilitate the analysis of these candidates. This will be essential to advance further in the understanding of the mechanisms involved in disease, and also to qualify the biomarker utility of the target proteins [40].

Regarding the MS pillar, the development of selected/multiple reaction monitoring (SRM/MRM) in the context of the HPP [41], and its increasingly frequent application in clinical studies [42] is a highly valuable tool for alleviating the existing bottleneck in moving markers from discovery phases to the clinical arena. In fact, targeted analyses based on this promising technology have recently begun to be conducted for assessing the levels of specific proteins in observational OA cohorts [43].

Table 2 – Top 10 proteins detected as altered in the proteomic analyses performed by our group for the search of osteoarthritis biomarkers.

Acc. no.	Protein name	Chondrocytes [19]	Cartilage [26]	Synovial fluid [31]	Serum [30]
GO:0001501 Skeletal system development					
P16112	Aggrecan core protein	Down	Down	ND	Up
P49747	Cartilage oligomeric matrix protein	ND	Up	Up	Up
P05452	Tetranectin	Down	Up	Up	Up
GO:0051216 Cartilage development					
P51884	Lumican	Up	Up	ND	Up
P36222	Chitinase-3-like protein 1 (YKL-40)	Up	Up	ND	Up
GO:0007155 Cell adhesion					
P07996	Thrombospondin-1	Up	ND	ND	Up
P02751	Fibronectin	Up	Up	ND	Up
GO:0006955 Immune response					
Q92954	Proteoglycan 4	Up	Up	ND	Down
P00746	Complement factor D	ND	ND	Up	Up
GO:0050728 Inflammatory response					
P36955	Pigment epithelium-derived factor	ND	Up	ND	Up

Furthermore, efforts for the improvement of multiplexed, high-throughput technologies that can bridge the gap between basic research and in vitro diagnostic methods have also been made in the area of affinity proteomics (AB pillar), especially with the development of protein arrays and other antibody-based profiling studies [44]. Different screening strategies using protein arrays have been already applied for profiling autoantibodies, cytokines, and bone-turnover products in rheumatoid arthritis [45]. In osteoarthritis, we have carried out a very recent study employing antigen microarrays and NAPPA (nucleic acid programmable protein arrays) to characterize the differential autoantibody profiles in samples from OA, rheumatoid arthritis (RA) and healthy controls [46]. At present, further studies are being performed using antibody arrays for the screening of larger cohorts in order to verify putative OA biomarker candidates (Lourido et al., unpublished results).

Finally, the bioinformatics-driven knowledgebase (KB) pillar of HPP draws upon resources that already exist such as SwissProt/Uniprot, PRIDE, Peptide Atlas or Human Protein Atlas databases. Although KB resources are continuously being enhanced, there is still much room for improvement in the area of rheumatic diseases. As an example of this, the three tissues that are most affected in this kind of pathologies: cartilage, synovium and bone, have not yet been explored in the Human Protein Atlas initiative [47]. Therefore, our research group is developing an initiative within our hospital (DIPROA project) in order to join the proteomics data that have been acquired after more than ten years of investigation on joint tissues together with all the clinical data from the patients. All this information is being employed to build a business intelligence (BI) system combining data warehousing, online analytical processing (OLAP) and knowledge discovery by data mining (DM). This will be a highly valuable tool for knowledge management, allowing innovation in the health care practice that is currently being carried out in the Rheumatology Services.

7. Conclusions

The B/D platform of the Spanish HPP consortium is the core for the development of proteomic studies on cancer, obesity, cardiovascular, infectious and rheumatic diseases. The initiative on rheumatic diseases will apply its experience in the study of joint cells, tissues and body fluids for improving osteoarthritis research on the three pillars of the HPP project, which will be undoubtedly useful for addressing the important unmet clinical needs of this disease and other rheumatic pathologies.

Transparency Document

The [Transparency document](#) associated with this article can be found, in the online version.

Conflict of interest disclosure

The authors declare no competing financial interest.

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